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Morphological and anatomical characters of apple leaves associated with cultivar susceptibility to spider mite infestation

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Abstract

Morphological and anatomical characters of apple leaves associated with cultivar susceptibility to two-spotted spider mite (*Tetranychus urticae* (Koch.)) infestation were investigated. The following six apple cultivars were selected for the research: Close, Lobo (suceptible) and Katja, Piros, Jester, Marvit (more resistant to mites infestation). The cultivars of a relatively lower susceptibility, (except Marvit) were provided with a poorer pubescence on abaxial epidermis of foliage leaf than those susceptible. There was no strict correlation between stomata number per unit of the leaf surface and the susceptibility to *T. urticae* infestation. A tight cell arrangement in the spongy mesophyll was particularly characteristic for cv Marvit, whereas in the leaves of cvs Close and Lobo this tissue was definitely loose. It seems that loose spongy mesophyll structure may facilitate feeding of mites.The results regarding the analysis of the lower (abaxial) epidermis of the leaves seem to suggest that the layout and thickness of cuticle, rather than the epidermal thickness itself could be a factor determining the susceptibility of apple cultivars to *T.urcitae*.

INTRODUCTION

Spider mites are common pests of various crops, including apple orchards, often occurring in very high numbers. There are several reasons for their remarkable potential, which is mainly determined by biological attributes of these pests such as high fecundity and multi-generation development over a vegetation season. Also, human activity with the excessive use of chemicals considerably contributes to such a state, since non-selective pesticides, frequently applied in orchards, significantly eliminate the beneficial fauna, causing the disturbance of the biological balance and spider mite outbreaks. Besides, these pests are well known for their ability to develop strains resistant to used chemical agents, therefore a constant search for new, often expensive pesticides is a necessity.

There are various approaches to solve the problem of spider mites in apple orchards, such as rotated use of selective acaricides, introduction of natural enemies, or fruit production based on cultivars resistant to these pests.

Considerable differences in the intensity of spider mite infestation have been frequently observed among apple cultivars in orchards. In Poland, this problem was investigated by Golik (1975), Dąbrowski and Rejman (1975), Skorupska and Kozłowski (1993), Anasiewicz and Winiarska (1993), and recently by Warabieda and Olszak (1994).

A low susceptibility to spider mites may come from the passive resistance of plant cultivar, related to the specific structure of leaves that disturbs the pest feeding and development. Such a resistance may be determined by both morphological characters of the leaf surface and its anatomical/cytological structure. In apple foliage, it can apply to the length and distribution of trichomes covering the lower epidermis (Goonewardene et al. 1976), thickness of cuticle and epidermis, stomata number, cell organization in the spongy mesophyll, or the content of phenolic compounds affecting the female fecundity and survival of spider mite larvae (Bielak 1979).

Also, the active resistance of plant cultivar may determine its susceptibility to spider mite infestation. In this case, the feeding pest induces specific metabolic changes in the leaves, which produce substances limiting or preventing from its attack and development (Kiełkiewicz 1995).

Most of the research on the resistance of apple cultivars to spider mites dealt with *Panonychus ulmi*. However, features of the leaf structure determining both types of the resistance to this pest, may appear less significant, or even entirely different in the case of other spider mite species. In particular, it concerns the two-spotted spider mite (*Tetranychus urticae*), that has evidently expanded over recent years within orchards in many regions of Poland. Thus, research on the mechanisms of apple cultivar resistance to this pest seemed to be highly required. Our former study showed differences among the cultivars with respect to the rate of *T.urticae* infestation. Some of them (Close, Lobo) were especially heavily attacked, while the other group of cultivars (Piros, Jester, Katja) was relatively spared by this pest (Warabieda and Olszak 1994). In consequence, it seemed worth-while to analyse the susceptibility of some apple cultivars to *T. urticae* infestation in relation to genetically determined, morphological and anatomical features of their leaves.

MATERIAL AND METHODS

The following six apple cultivars were selected for the research: Close (C), Katja (K), Piros (P), Jester (J), Lobo (L) and Marvit (M). Leaves for the analyses were collected on July 2, 1996, from 3 trees of each cultivar. An analysed sample consisted of 12 leaves per cultivar, obtained from dwarf shoots.

The analyses included:

1. Micromorphology of the leaves surface – structure of epidermal tissue (trichomes, stomata, cuticle or wax cover).

2. Internal structure of the leaves (thickness of lower –abaxial epidermis, spongy mesophyll, palisade mesophyll together with upper epidermis) and also the morphology and cell organization within particular tissues –adaxial.

The study was carried out with the use of a scanning electron microscope (SEM) and a light microscope (LM).

Preparation procedures for microscopic analyses:

1. SEM examination

Leaves sections obtained from the central part of a lamina (together with a part of the main vein) were fixed in CrAF agent (chromic acid, acetic acid, formalin), dehydrated in ethanol and acetone, than dried in liquid $C0_2$ at the critical point and covered with gold palladium. This material was examined and photographed with the JEOL scanning microscope of SM1 type.

2. LM examination

a) Anatomical microscopic sections

Leaves sections fixed and dehydrated (as described above) were dipped in paraffin, then cut across and stained with safranine and light green (Gerlach 1972).

b) Stomata count

The lower epidermis was isolated with cellotape, stained with toluidine blue and fixed in glycerol (Dyki and Habdas 1996). Number of stomata was always determined in the fixed field of view of a light microscope at the magnification of (25 x 12.5).

Observations of leaves sections and number of stomata were performed using the NU1 Zeiss microscope.

Results of the measurements were processed upon the analysis of variance, and the Duncan's Test was used for determining the significance of differences at P=0.05.

RESULTS

In all the examined cultivars, the leaves obtained from dwarf shoots were provided with superficial long, thin trichomes. Depending on their distribution, the trichomes twisted themselves to various degrees (Fig. 1). They were mainly formed by the epidermis covering the vascular bundles (veins), having also been scarcely distributed among the other cells. The density of this pubescence varied between the cultivars (Fig.1). A significantly lower density of the trichomes was found on abaxial epidermis of foliage leaf in cvs Katja, Piros and Jester as compared to Marvit, Close and Lobo.

In all the examined cultivars, the epidermis was covered with a layer of cuticle of a considerable thickness, that was also found on the surface of trichomes and stomatal cells (Fig. 2, 3). There were cracks of different shape and size within cuticle, particularly dense in cv Jester (Fig. 3). In all the cultivars, cuticle was also folded and wrinkled (Fig. 3).

Opposite to cv Lobo, Katja showed the most wrinkled surface of cuticle on the leaves. (Fig. 3). Under the cuticle-covered epidermis the analysis revealed the contour of lobar cells with folded tangent walls and various convexity. The stomata

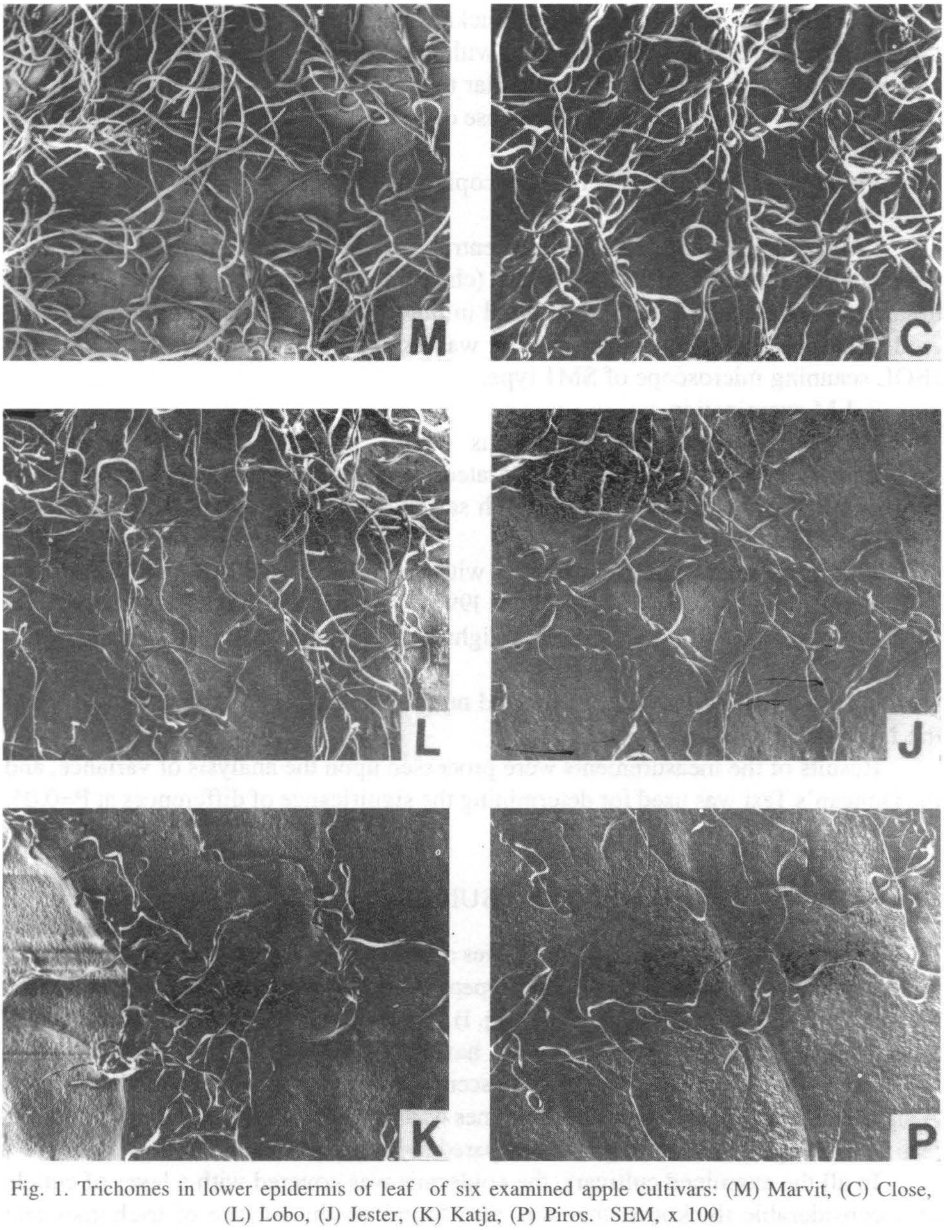

Fig. 1. Trichomes in lower epidermis of leaf of six examined apple cultivars: (M) Marvit, (C) Close, (L) Lobo, (J) Jester, (K) Katja, (P) Piros. SEM, x 100

located among these cells varied in shape, size and degree of arching of their guard cells (Fig.2, 3). In all the cultivars, stomata of different sizes were found within the same leaf (Fig. 2, 3).

Stomata numbers per 1 mm^2 on the lower epidermis of foliage leaf significantly differed among the cultivars. The stomata found in the dwarf-shoot leaves of cv

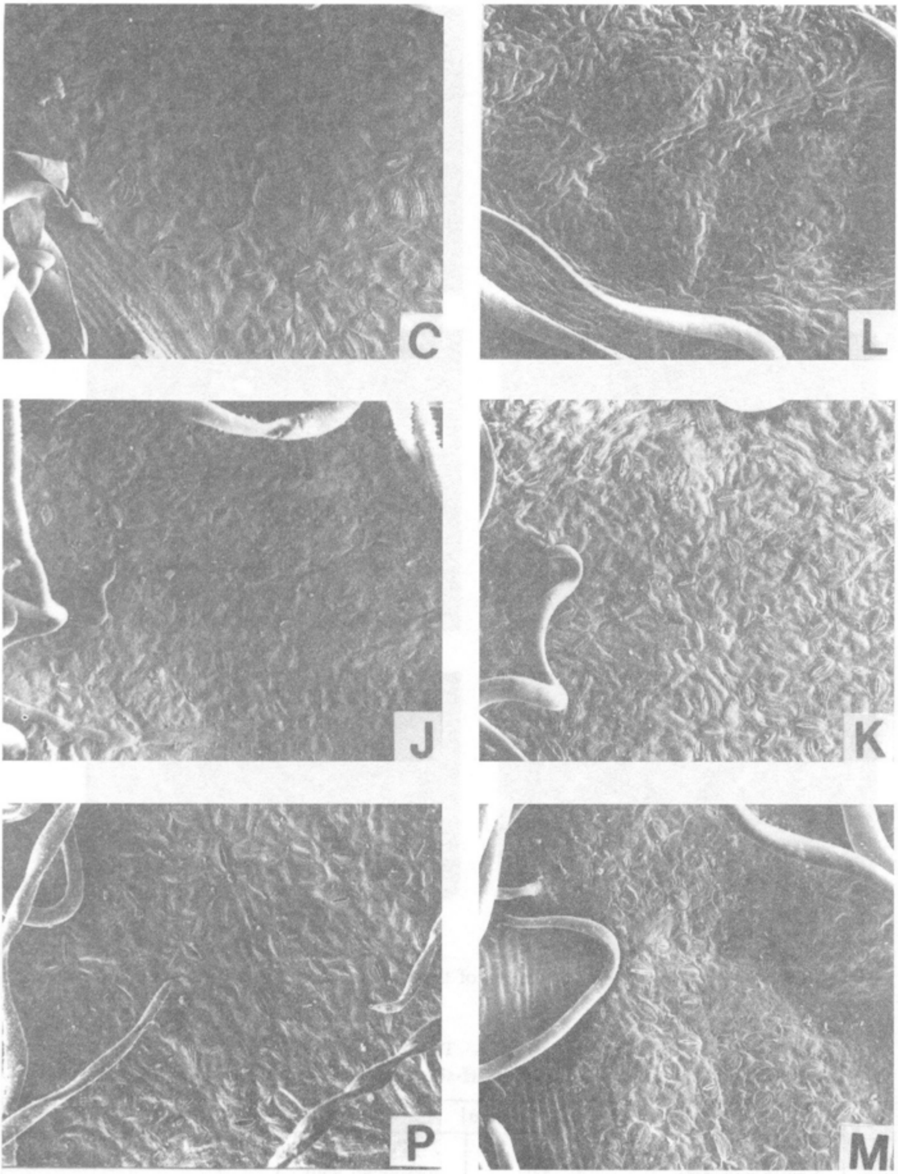

Fig. 2. Stomata in lower epidermis of leaves SEM, x 670. Notations as in Fig. 1.

Jester almost twice outnumbered those in cv Close. Also, cv Marvit was provided with numerous stomata. (Table 1).

The analyses of cross-sections obtained from the leaves revealed the differences among the cultivars with respect to the structure of the lower epidermis and mesophyll tissues. Cuticle on the external wall of the lower epidermis was thick and more distinct in cvs Jester, Marvit and Katja, as compared to other cultivars. Cuticle

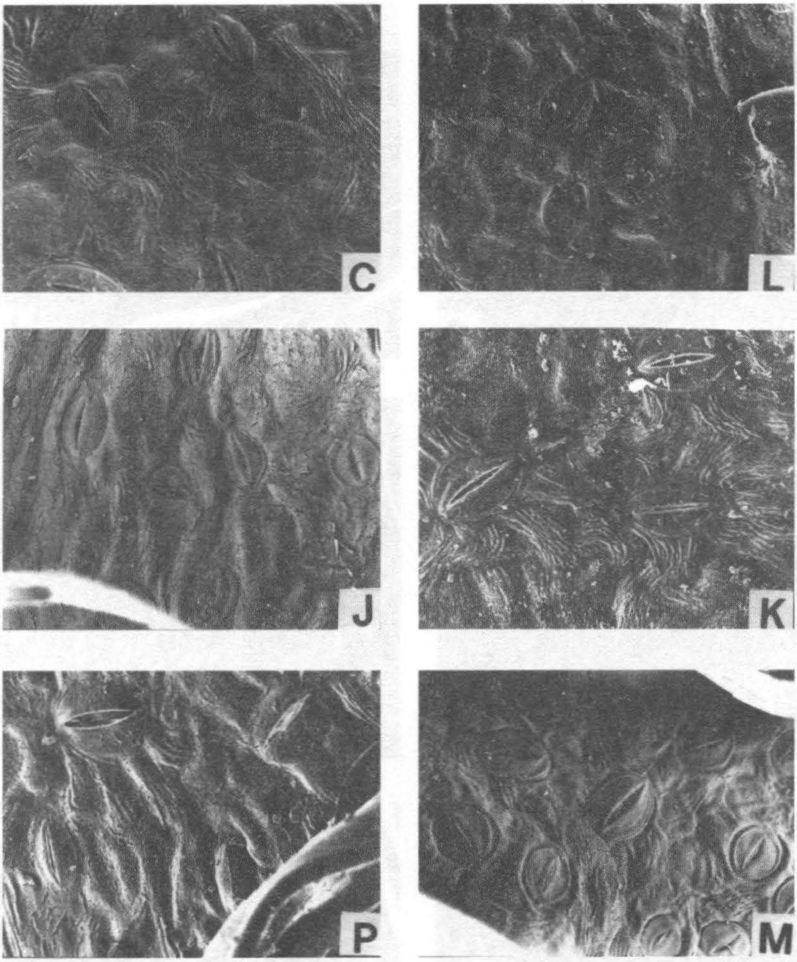

Fig. 3. Micromorphology of the lower epidermis of the leaves. SEM, x 1950. Notations as in Fig. 1.

Table 1 – Tabela 1

Tissue thicknes and stomata density dwarf-shoot leaves of examined apple cultivars

Cultivar	Tissue thickness [µm]			Stomata number per mm² on dwarf-shoot leaves
	Lower epidermis	Spongy mesophyll	Palisade mesophyll with upper epidermis	
Close	11.29 d	107.1 d	157.9 e	220 a
Lobo	11.08 d	82.3 b	150.9 de	310 c
Jester	10.06 c	92.1 c	141.6 cd	424 e
Katja	9.85 c	94.3 c	129.0 bc	281 b
Piros	9.28 b	104.6 d	114.0 a	268 b
Marvit	8.62 a	69.9 a	119.5 ab	341 d

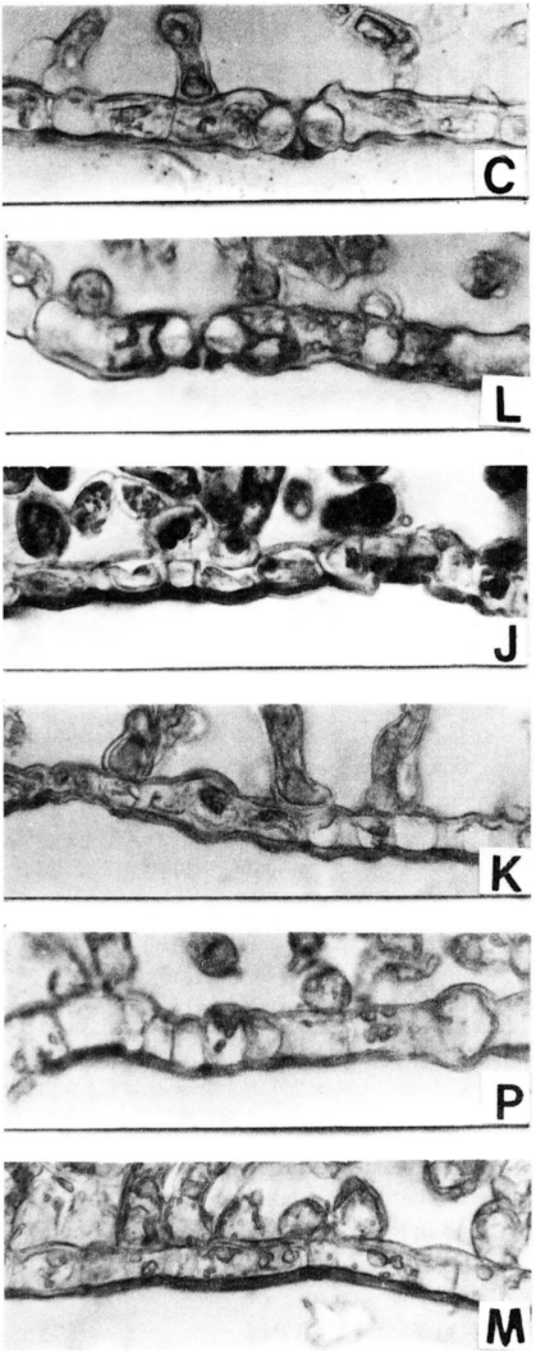

Fig. 4. Cross-sections through lower epidermis of the leaves and the layer of cuticle covering it. LM, x 650. Notations as in Fig.1.

was also marked on the inner wall of the epidermis bordering with the spongy mesophyll, that was particularly visible in cv Katia (Fig.4). Irrespective of the cultivar, the basal cells of a trichome were provided with a distinctly thicker cuticle layer than other epidermal cells (Fig. 5). Contrary to cvs Katja, Jester, Piros and Marvit, cuticle covering stomata was poorly marked in cvs Close and Lobo (Fig. 4).

Cells of the lower epidermis, differing in size, were strongly vacuolized and arranged into a compact, single-layered system in a cross-section (Fig. 4). A tight cell arrangement in the spongy mesophyll was particularly characteristic for cv Marvit, whereas in the leaves of cvs Close and Lobo this tissue was definitely loose. Besides, cv Marvit was provided with more numerous vascular bundles than other cultivars, what was visible in the cross-sections at the same microscopic magnification (Fig. 6).

In all the cultivars, the palisade mesophyll consisted of 2-3 layers of cells varying in their length and width from the smallest in cvs Marvit and Jester to the distinctly larger dimensions in Close and Lobo (Fig. 6).

The examined cultivars significantly differed in the thickness of particular cell layers, i.e. lower epidermis, spongy mesophyll, palisade mesophyll together with upper epidermis. The lower epidermis was thicker in Lobo and Close as compared to other cultivars

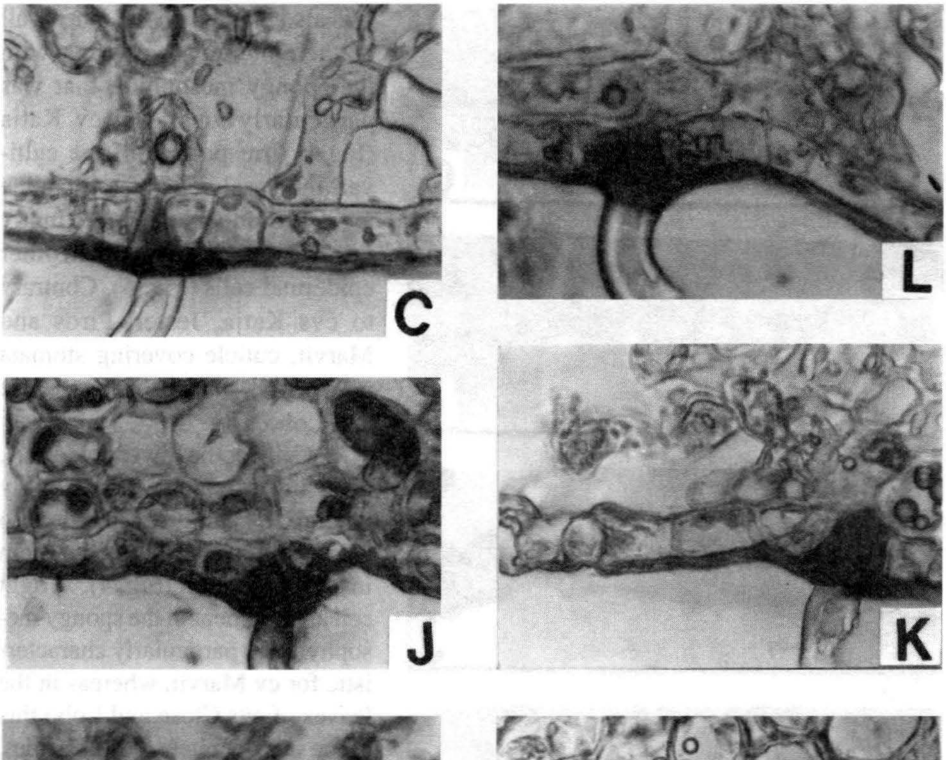

Fig. 5. Cross-sections through the lower epidermis and spongy mesophyll showing the thickness of the cuticle layer covering the basal cells of trichomes. LM, x 750. Notations as in Fig. 1.

(Table 1). The spongy mesophyll was the thinnest in Marvit and Lobo leaves, and significantly thicker in cvs Piros and Close. The thickest layer of palisade mesophyll together with the upper epidermis was found in the foliage of cv Close and Lobo.

DISCUSSION AND CONCLUSIONS

The research proved that there were differences in the morphological and anatomical structure of the leaves among the examined cultivars. The pubescence on the

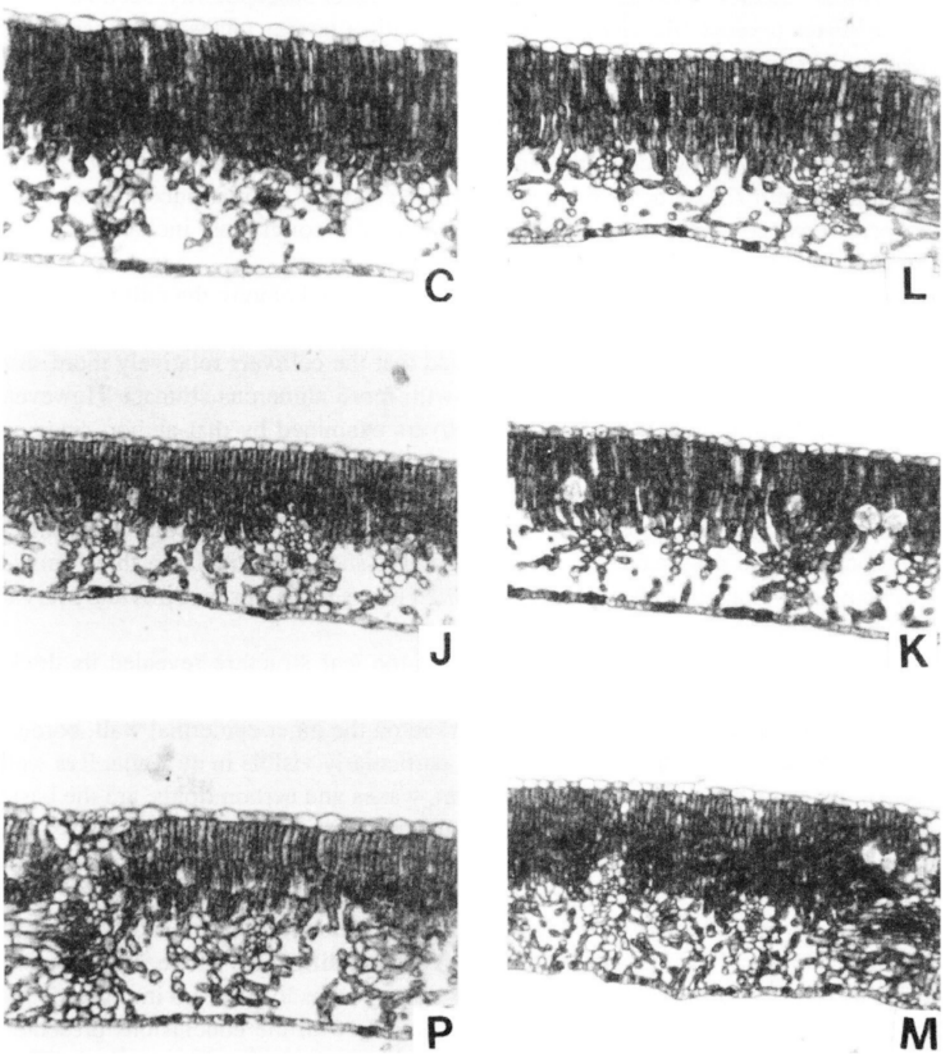

Fig. 6. Cross-sections through the apple leaves. LM, x 100. Notations as in Fig. 1.

leaf surface is one of the factors that may affect the development of spider mite populations, however, this is still a subject of discussion. According to Bielak (1979), a dense pubescence on the lower epidermis of foliage leaf may be one of the reasons that cv Jonathan is barely infested with *P.ulmi.* However, Goonevardene et al. (1976) observed the most substantial populations of this species on apple seedlings with leaves covered with a dense pubescence. Kamel and Elkassaby (1965) while testing the susceptibility of various cotton cultivars to *Tetranychus telarius*, found the lowest infestation with this pest in a cultivar with leaves covered with dense trichomes. In the present research, the cultivars of a relatively lower susceptibility, such as Piros, Katja, Jester (except Marvit) were provided with a poorer pubescence than those susceptible (Lobo, Close). However, it should be assumed that a very dense pubescence would prevent from spider mites by limiting their access to epidermal cells and making their feeding more difficult. This factor could be essential in the case of *P. ulmi*, the body of which is provided with very long setae, but it may be of no importance with *T. urticae* whose setae are much shorter. A foliar pubescence seems rather to protect a pest from adverse environmental conditions, including certain predators and the effect of pesticides.

Stomata number per unit of the leaf surface varied among the cultivars, however, there was no strict correlation between this factor and the susceptibility to *T. urticae* infestation. Bielak (1979) observed that the cultivars relatively more susceptible to *P. ulmi* were usually provided with more numerous stomata. However, such a correlation did not apply to all cultivars examined by that author, since cv Marvit (barely infested with spider mites) was rich in the stomata. It was also showed by our study that cv Close (susceptible to *T. urticae*) was provided with the least numerous stomata. The present research did not confirm the thesis that the stomata may facilitate the pest access to the mesophyll tissue. In particular, a thick cuticle layer covering stomatal cells, that was observed in cvs Katja, Jester, Piros and Marvit, may be another obstacle for mites feeding.

Referring to cuticle, the examination of the leaf structure revealed its thick, distinctly marked layer on the external wall of the epidermis in cvs Jester, Marvit and Katja. The presence of cuticle was also marked on the inner epidermal wall, bordering with the spongy mesophyll, which was particularly visible in cv Katja. It is well known that such substances as cutin, suberin, waxes and certain lipids, are the basic constituents of cuticle chemical composition, being responsible for its hardness. So, it seems that a double-layered cuticle is an essential barrier for spider mite chelicerae.

The lower epidermis varied in the thickness among the examined cultivars at a statistically significant level, however, the relevant difference between the two extreme cultivars was only 2.7 μm. Thus, this factor seems to be of no importance for the development of spider mites. This corresponds with the conclusions presented by Kiełkiewicz and Van de Vrie (1983), following their study of anatomical differences in the leaf structure of two strawberry cultivars, which varied in the resistance to *T. urticae*.

The results regarding the analysis of the lower epidermis seem to suggest that the distribution and thickness of cuticle, rather than the epidermal thickness itself could be a factor determining the susceptibility of apple cultivars to *T.urcitae*.

According to Avery and Briggs (1968), leaf tissue injuries due to spider mite feeding are usually concentrated near the vascular bundles, which has been confirmed by our research (results to be published). It seems that the phloem-associated cells may be an especially attractive source of nutrients, since they show a high level of metabolism and an increased content of both proteins and carbohydrates. To reach those cells, the stylets of spider mite chelicerae must penetrate through a layer of the spongy mesophyll. The thickness of this tissue should not be an obstacle for the pest, as it appears from the present research, but its structural cohesion (cell size and number as well as intercellular air spaces) may be an essential factor. As we observed in the leaves of cv Marvit in particular and also of other cultivars barely infested with spider mites (Piros, Jester and Katja), their mesophyll was more coherent as compared to cvs Close, Lobo. This does not correspond with the results of other research (Bielak 1979) that revealed the presence of small intercellular spaces within the spongy mesophyll in the leaves of cultivars relatively more susceptible to *P. ulmi*.

It seems that certain contradictions in the literature data, regarding the influence of the leaf structure of a host plant on the development of spider mite populations, could be a result of an interaction between a particular pest species and some characters of the leaves. The two-spotted spider mite, as a polyphagous pest attacking over 300 plant species, has developed efficient adaptive mechanisms. Morphological and anatomical characters of the leaves are not the only factors that may determine the resistance of a particular plant species/cultivar to this pest. Another source of such resistance apple cultivars could come from the occurrence of antibiosis towards the spider mites (including *T.urticae*), brought about e.g. by phenolic substances in the leaves (Warabieda and Olszak 1995). However, a comparative cytological examination of both healthy and infested leaves is required to draw up the final conclusions on sources of different susceptibility to *T. urticae* among various apple cultivars.

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Morfologiczne i anatomiczne cechy liści wybranych odmian jabłoni, związane z ich podatnością na zasiedlenie przez przędziorki

Streszczenie

Celem pracy było określenie związku pomiędzy morfologicznymi i anatomicznymi cechami liści jabłoni, a ich podatnością na przędziorka chmielowca (*Tetranychus urticae* (Koch.). Do badań wybrano sześć odmian jabłoni: Close, Lobo (podatne) oraz Katja, Piros, Jester, Marvit (odporniejsze na zasiedlenie przez przędziorki). Odmiany mniej wrażliwe, (z wyjątkiem odmiany Marvit), miały słabiej rozwinięty kutner na dolnej stronie liścia niż odmiany podatne. Nie zaobserwowano związku pomiędzy liczbą aparatów szparkowych na jednostkę powierzchni blaszki liściowej a wrażliwością na porażenie przez *T. urticae*. Tkanka miękiszu gąbczastego liści odmiany Marvit, charakteryzowała się większą zwartością, podczas gdy najluźniejszy układ tych komórek znajdowano w liściach odmian Close i Lobo. Oznacza to, że luźna struktura miękiszu gąbczastego może być czynnikiem ułatwiającym żerowanie przędziorków. Wyniki dotyczące analizy dolnej epidermy liści wydają się sugerować, że raczej rozmieszczenie i grubość kutikuli, niż grubość samej epidermy może być czynnikiem wpływającym na wrażliwość odmian jabłoni na *T. urticae*.